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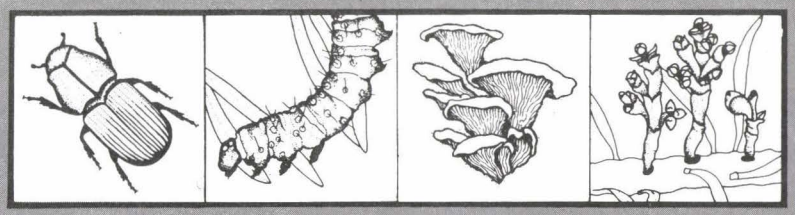

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PATHOGENICITY OF *ALTERNARIA ALTERNATA* ON YOUNG DOUGLAS-FIR AND ENGELMANN SPRUCE GERMLINGS

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INTRODUCTION

Isolations from bare-root Douglas-fir (*Pseudotsuga menziesii* Dougl.) seedlings with basal swellings that had been outplanted at the Priest River Experimental Forest yielded primarily *Alternaria alternata* (Fr.) Keissler (James 1985). All symptomatic seedlings yielded this fungus at the swelling site and within roots. Although species of *Alternaria* have been implicated in several plant diseases (Hepting 1971; Mark et al. 1976; Vaartaja and Crum 1956), these soil-borne organisms are often considered to be saprophytic on a number of different substrates. Therefore, to assess the role of isolates of *A. alternata* in causing diseases of young conifer seedlings, pathogenicity tests were conducted.

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MATERIALS AND METHODS

Douglas-fir and Engelmann spruce (*Picea engelmanni* Parry) were the two coniferous species selected for evaluation. Two groups of seedlings were chosen for inoculations. The first were young germlings 8 weeks old and the second were seedlings 14 months old. All seedlings had been grown within small Leach® containers in standard peat-vermiculite soil mix. Two inoculation techniques were used. The first entailed spraying foliage of seedlings with a conidial suspension (8.25 X 10 6/ spores/ml) of an isolate of *A. alternata* obtained from a Douglas-fir seedling with basal swelling. Spores were harvested from 14 day-old cultures grown on potato dextrose agar (PDA) and mixed with sterile distilled water. Inoculated seedlings were sprayed to runoff with the conidial suspension. Seedlings were then placed in a moist chamber (similar to that used to inoculate seedlings with *Cronartium ribicola* (Fisch.) (Hoff 1983) for 48 hours before being replaced on greenhouse benches. Two hundred germlings and 200 seedlings were inoculated for each species.

The second inoculation technique was similar to that used to inoculate conifer seedlings with *Fusarium* spp. (James and Gilligan 1984). Inoculum was prepared as described by Miles and Wilcoxson (1984). Inoculum was produced in galvanized metal pans (5 x 25 x 35 cm) lined with a double layer of aluminum foil. Perlite, an inert, inorganic, siliceous rock of volcanic origin commonly used in potting mixtures, was the matrix for fungal growth. In each metal pan, 150 g of yellow cornmeal was moistened with 300 ml warm 1 percent PDA and left standing for 15 minutes, then 75 g of perlite was thoroughly mixed with the cornmeal. The pans were covered with aluminum foil and autoclaved for 60 min. at 121 degrees C. After cooling, the perlite-cornmeal cake was inoculated with 1 cm square pieces of mycelium from 14 day-old *A. alternata* cultures grown on PDA. Fifty ml of sterile, distilled water were added after mixing and the pan was sealed. Closed pans were incubated in the dark at about 24 degrees C for 24 days. Fungal cake mixtures were air dried on a tabletop for 3 days and stored in plastic bags at 8 degrees C until needed.

Twenty-four to forty germlings or seedlings were inoculated with the perlite-*Alternaria* inoculum for each species. Germlings and seedlings were carefully removed from their containers so that roots were not damaged. Roots were then washed thoroughly under tap water to remove adhering soil and transplanted into other containers with the inoculum, mixed with autoclaved peat-vermiculite soil mix at specific concentrations. Concentrations of inoculum were determined on a weight/weight basis; three levels were used: 1:5 (20 gms of inoculum per 100 gms soil mix), 1:10 (10 gms of inoculum per 100 gms soil mix) and 1:20 (5 gms of inoculum per 100 gms soil mix). Controls consisted of mixing 100 gms autoclaved peat-vermiculite with 10 gms of uninoculated perlite.

After inoculation, seedlings were placed on greenhouse benches and watered when necessary. Periodic examinations for disease symptoms were made. When diseased seedlings were discovered, they were removed from containers and isolations made to determine if *A. alternata* was obtained from diseased tissues. Douglas-fir germlings were monitored for 12 weeks following inoculation; the larger Douglas-fir seedlings and both Engelmann spruce germlings and seedlings were monitored for 31 weeks.

Differences in germling mortality for the different inoculum concentrations were compared with an analysis of variance. Treatment (inoculation concentration) differences were located with Tukey's comparison test.

RESULTS AND DISCUSSION

None of the seedlings of either species inoculated with the *Alternaria* spore suspensions became diseased. Likewise, none of the older seedlings inoculated with the perlite inoculum became diseased. Only young germlings of each species which had been inoculated with the perlite inoculum became diseased. Results of these inoculations are summarized in table 1. In general, those seedlings inoculated with the highest concentration of inoculum (1:5) were killed. *Alternaria alternata* was the only organism consistently isolated from the roots of killed seedlings. However, many of the control germlings also died, especially Douglas-fir. *Alternaria* was likewise frequently isolated from the roots of these seedlings. Killed trees frequently had extensively decayed roots.

Table 1.--Mortality of Douglas-fir and Engelmann spruce germlings transplanted into soil infested with *Alternaria alternata*.

Inoculum concentration *1/*	Douglas-fir *2/*	Engelmann spruce *3/*
1:5	*4/*100.0 A	*4/*60.0 A
1:10	97.1 AB	16.6 B
1:20	65.7 B	4.2 B
Control	66.7 B	16.6 B

1/ On a w/w basis with autoclaved peat-vermiculite growing medium.

2/ Cumulative percent mortality 12 weeks after transplanting.

3/ Cumulative percent mortality 31 weeks after transplanting.

4/ Means followed by the same capital letter are not significantly different (P=0.05) using the Tukey's comparison test. All percentages underwent arc-sin transformations before subjected to statistical tests.

These results indicates that *A. alternata* was probably pathogenic to very young Douglas-fir and Engelmann spruce seedlings at high inoculum concentrations. However, relatively high rates of mortality of the control germlings, especially Douglas-fir, indicated that the transplanting procedures used were quite stressful to germlings. Also, the high populations of *A. alternaria* used in inoculations are probably not representative of the common situation in either forest or nursery soils. Also, basal swellings which were commonly associated with diseased seedlings in the field were not reproduced in our inoculation tests. Therefore, we conclude that if *A. alternata* was pathogenic to young conifer seedlings, it was very weakly so and probably required substantial environmental stress to render seedlings susceptible to infection and disease development.

We also conclude that the basal swellings found on seedlings in the field were probably not initiated by *Alternaria*, but rather by some environmental factor such as high surface soil temperatures. It was likely that *Alternaria* invaded tissue damaged by other factors and caused tissue degradation which probably accelerated seedling mortality. *Alternaria alternata* would probably not have caused disease without predisposition.

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